REMARKS/ARGUMENTS

I. Procedural History

The present paper is being submitted accompanying a Request for Continued Examination. Applicants received a Final Office Action in the instant application with a mailing date of December 13, 2001. In response, Applicants discussed the Final Office Action with the Examiner on May 31, 2002 and filed a Notice of Appeal on June 12, 2002. An Action from the Applicants is due September 12, 2002, with a one month extension of time.

Applicants' representative further discussed the Final Office Action with the Examiner in a telephone conference on August 30, 2002. During that conference, the Examiner advised that Applicants may proceed with the filing of Request for Continued Examination and an accompanying paper reiterating the Applicants' position with respect to the remaining rejection under 35 U.S.C. §103. The following remarks address the Examiner's concerns. Applicants believe the claims are in condition for allowance and respectfully request an early indication of such a favorable disposition of the case.

II. Status of Claims and Cancellation of Non-Elected Subject Matter.

Claims 1-42 were pending in the application at the time of Examination. Claims 39-42 were withdrawn from consideration as being directed to non-elected subject matter. Claims 1-38 were under examination and were rejected under 35 U.S.C. §103. Applicants traverse the rejection.

Claims 39-42 have been cancelled herein by amendment as being drawn to non-elected subject matter. This amendment is made without prejudice or disclaimer and Applicants reserve the right to prosecute these claims in subsequent continuing applications.

III. The rejection under 35 U.S.C. §103 should be withdrawn

In the Office Action, the Examiner reiterated the rejection of claims 1-38 as being rejected under 35 U.S.C. §103 as being unpatentable over Kangas *et al.* (*Med. Boil.* 62:338-343, 1984) in view of Redick *et al.* (*J. Biol. Chem.* 257:15200-3) and Connors *et al.* (*Biochem*

Pharmacol. 24:2217-2, 1975).

According to the Official Action, Kangas and Redick teach ATP assays, MTT assays, Alamar Blue assays and Rhodamine assays. The Examiner goes on to state that Kangas teach a method of predicting the cytotoxicity of a chemical compound by measuring a number of different assays and that Kangas teaches the treatment of cancer. The Examiner states that Redick teaches choosing liver cell lines and Connors teaches *in vivo* methods of screening for anti-cancer agents and predicting the cytotoxicity of chemical compounds. The Examiner asserts that "one of skill in the art would have been motivated to modify the teaching of Kangas by the addition of liver cell lines as taught by Redick and *in vivo* as taught by Connors" to facilitate the use of the techniques in real world pharmaceutical compositions instead of merely *in vitro* testing. Applicants traverse the rejection.

Three criteria must be met in order to establish a case of *prima facie* obviousness of a claimed invention in view of the cited art. It must be shown that:

1) The art contains a teaching of each of the elements of the claimed invention;

2) A given combination of references must provide some suggestion or motivation to modify the reference(s); and

3. There must be some reasonable expectation of the success of such modification of the reference.

The motivation and the reasonable expectation of success must come from the art and not from the Applicants' own disclosure. As stated in MPEP §2143, all three of the above criteria ***must*** be met in order to properly establish *prima facie* obviousness. ***None*** of these criteria are met by the combination of Kangas *et al.* in view of Redick *et al.* and Connors *et al.*cited by the Examiner.

The present invention is directed to an *in vitro* method of predicting the *in vivo* cytotoxicity of a chemical compound by performing multiple assays to develop a cytotoxicity profile for the chemical compound to accurately assess the cytotoxicity of the compound *in vivo*

(e.g., see page 23 of the specification). In particular, the invention involves conducting at least three assays with different biochemical endpoints in order to predict the *in vivo* toxicity of the compound. None of the references cited either alone, or in combination, provide such a teaching.

The teachings of Kangas are entirely directed to evaluating a method of using ATP to measure the viability of a cell. Kangas describes the validation of an ATP bioluminescence assay by contrasting the results of the ATP assay ***individually*** with the assays for cell number, viability, thymidine incorporation and stem cell assay. However, at no point does Kangas teach or suggest that it would be desirable or possible to better predict the *in vivo* cytotoxicity of an agent by performing at least three of these assays, determining a C_{tox} concentrations from a combination of these assays and generating a cytotoxicity profile of a cytotoxic agent as is taught by the present invention.

The entire focus of Kangas is to produce one ". . .universal method-applicable to any cell line. . .to enable the evaluation of cell growth as well as cell death." (See page 338 first full paragraph under INTRODUCTION). Thus, rather than teaching the use of ***multiple assays*** to produce a cytotoxicity profile of a cytotoxic agent (as the present invention contemplates), Kangas is aiming to produce one uniform assay in which ***only one*** parameter *i.e.* bioluminescence of cellular ATP, is measured. In effect, Kangas is teaching away from the present invention and proposes that ". . .the ATP-bioluminescence method ***is a powerful alternative*** to any other cell growth estimation method in vitro. . ." (***emphasis added***; Kangas Abstract, page 338).

Neither the Redick nor the Connors reference does anything to rehabilitate the flaws of Kangas. Redick does not teach an *in vitro* GST leakage monitoring assay in which the leakage of GST into the media of a cell culture is determined, but rather reports the immunohistochemical localization of three isozymes of GST, transferases B, C, and E, in the liver of the rat using sheep antibodies raised against these three isozymes of hepatic GST. Hence, this reference provides background for determining the presence of GST on a histochemical slide and suggests that this enzyme may be used a marker for liver damage, in tissue sections. Redick ***does not*** provide any teaching or direction of monitoring GST leakage

into cell culture media. As such, the combination of Kangas and Redick still do not provide the requisite elements of claim 1 in that it does not provide a teaching of conducting at least three assays to determine the C_{tox} of an agent.

The Connors *et al.* reference points out various limitations of applying *in vitro* assays to test for and predict the *in vivo* cytotoxicity of agents. According to the Connors reference, *in vitro* tests are of limited use in such an endeavor. Connors focuses on determining the cytotoxic effects of an agent in single assays, and does not provide any teaching or suggestion for using the composite data generated through the use of multiple assays assessing the effects of an agent on a plurality of parameters as is required by the claims of the present invention. Connors proposes that cytotoxicity should be tested ***in vivo using animal models***. The present invention overcomes ineffectiveness of single assays in predicting the cytotoxicity discussed in Connors by using the ***in vitro cytotoxicity screens*** of the present invention. Moreover, the cytotoxicity screens of the present invention alleviates the problems of expense and difficulty of obtaining animal models (see specification page 1-2 for discussion of the drawbacks of using animal models for initial cytotoxicity screening.) The combination of Kangas, Redick and Connors fails to teach or suggest that it is either desirable or even possible to predict *in vivo* cytotoxicity using a cluster of at least three *in vitro* assays. Hence, the cited references do not provide a teaching of all the elements of the claimed methods.

Moreover, the Connors reference, on its face, disparages the usefulness of in vitro assays for such predictions and suggests instead that one of skill in the art should employ in vivo assays. In addition, the Kangas reference specifically teaches that the cytotoxicity of agents should be assessed using only one uniform assay in which ***only one*** parameter *i.e.* bioluminescence of cellular ATP, is measured. Thus, not only does the combination of all the references not teach all the claimed elements, two out of the three references cited actually teach away from the present invention. As such, viewing these references in combination, one of skill in the art would not be motivated to conduct multiple *in vitro* assays in order to predict *in vivo* cytotoxicity of an agent.

Furthermore, even if one of skill in the art were to combine all the references and fortuitously conduct a method of the present invention using at least three *in vitro* assays

simultaneously to predict the *in vivo* cytotoxicity of a given agent, that individual would still not have a reasonable expectation of success of achieving such a result because at least two of the references actually teach away from such a result. The Examiner has failed to cite any basis in the prior art for suggesting that the Applicants' invention could achieve the excellent results that it does in predicting the *in vivo* cytotoxicity of an agent using a cluster of *in vitro* assays.

In summary, the cited references, neither alone nor in combination, teach or suggest all the elements of Claim 1; there is no motivation or suggestion to combine the teachings of the cited references and even if one of skill in the art were to combine the references, there would be no reasonable expectation of success of achieving the claimed invention. The references do not establish the alleged *prima facie* obviousness of the invention of Claim 1.

Claims 2-38 ultimately all depend from Claim 1 and thus are also patentable over the cited references. The dependent claims are directed to numerous additional aspects and further define the invention claimed in claim 1. The Examiner has not cited and Applicants see no evidence of a teaching of identifying the NOEL concentrations which is the highest concentration of the chemical compound at which a measurable toxic effect of the chemical is not observable (required element of claim 3); determining a TC_{50} for each of the indicators of cell health (required element of claim 4); a method of identifying a lead compound for drug development (claim 31) or numerous other features of the other dependent claims. Such teachings must come from the prior art and must not be generated through a hindsight construction following the teachings of the present invention. In view of the foregoing, Applicants respectfully request that the rejections of Claims 1-38 under 35 U.S.C. §103(a) over Kangas *et al.* in view of Redick *et al.* and Connors *et al.* be withdrawn.

In view of the above remarks, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to withdraw the outstanding rejection of the claims and to pass this application to issue.

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Respectfully submitted,

By _____

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